# Some Minor Components of Casein and Other Phosphoproteins in Milk. A Review



**Abstract**

γ-Casein and other minor proteins, tentatively designated TS-, R-, and S-casein, have been identified in unfractionated casein by disc gel electrophoresis. γ-Casein is polymorphic and like β-casein it shows three variants of the A type and one B type. So far, no γ-C casein has been observed. The $A^2$ and B polymorphs of γ- and β-casein show the same amino acid substitutions. The R-casein is found in A samples; the S-casein is associated with caseins typed B with respect to γ-, β-casein. TS-Caseins occur in all samples and are polymorphic. This review will also discuss recent data on other minor caseins, the components of the proteose-peptone fraction, and the phosphoglycoprotein fraction of whey.

Casein has been defined as that protein fraction precipitated by acidifying raw skimmilk to pH 4.6 to 4.7 (18). More recently, this definition has been modified so that casein is described as a heterogeneous group of phosphoproteins precipitated from skimmilk at pH 4.6 and 20 C (38). Casein exists in milk in complex particles (micelles) containing calcium and phosphate, which can be sedimentated by ultracentrifugation. It is known that the micelles are stabilized by unique complexes of κ-casein with $\alpha_s$- and β-casein (33, 41, 42). These three proteins comprise most of the casein in the micelle, yet there are a number of other caseins present in the micelle which also precipitate with the casein on acidification of milk. Whether these minor components are an essential part of the complex has not been established; however, a number of them have been isolated. Not all of the phosphoproteins in milk are precipitated at pH 4.6 with the casein. It appears that some remain in solution while others are distributed between the casein and whey fractions. In this review the more recent studies on these minor caseins and other phosphoproteins will be discussed.

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Wake and Baldwin (40), using starch gel electrophoresis in urea at an alkaline pH, demonstrated that the pattern for casein contains about 20 protein bands, among which the α-casein [now called $\alpha_{s1}$-casein (35)] and β-casein bands are most prominent. Because casein from pooled skimmilk was used, some of these bands probably represented polymorphs, particularly those of the β- and $\alpha_{s1}$-caseins. They also found that κ-casein shows a broad staining area of slower mobility than that of β- and $\alpha_{s1}$-casein. The other bands represent the minor proteins found in the casein fraction. Numbers were assigned to each band depending on its position relative to a zone designated as 1.00 which had a mobility slightly slower than that of $\alpha_{s1}$-casein. Using this system and depending on the genetic types, the $\alpha_{s1}$-caseins were found to range from 1.07 to 1.18 and β-caseins from 0.70 to 0.80 (38). Some of the slow-moving components, identified as Bands 0.18, 0.34, and 0.41, were referred to as the γ-casein fraction (26). These bands were representative of the first fraction of reduced and alkylated casein eluted from a DEAE-cellulose column at pH 7.0 with a 2.0 M borate, 0.18 M phosphate buffer, containing 20% dimethylformamide. This fraction also showed an electrophoretic band corresponding to one of the reduced and alkylated κ-caseins, together with some material which moved toward the cathode. One of the protein bands, Number 0.86, with a mobility between $\alpha_{s1}$- and β-casein, disappeared on reduction and alkylation of the casein.

*γ-Caseins.* Mellander (28) observed that casein is composed of three electrophoretic components, which he named α-, β-, and γ-casein in the order of decreasing mobility in moving-boundary electrophoresis. Hipp et al. (14, 15) isolated γ-casein by chemical fractionation and estimated that it amounts to about 3% of the unfractionated casein. They also found a considerable amount of material associated with the γ-casein fraction with a mobility slower than that of γ-casein. Although the purified γ-casein showed a single electrophoretic component at pH 8.6, as determined by moving-boundary electrophoresis, it proba-

bly contained other protein impurities of slightly different mobilities.

The isolation of $\gamma$-casein using column chromatography has also been described (13). Chromatography on DEAE-cellulose of that part of acid-precipitated casein extracted by dilute acid at 3 C yielded a protein eluted from the column with the starting buffer of 0.005 M phosphate, pH 8.3. It was designated temperature-sensitive (TS), because it gave a clear solution at pH 8, 3 C, but became turbid with warming. The $\gamma$-casein was eluted at 0.02 M phosphate, pH 8.3. The $\gamma$-casein purified by this method showed physical and chemical properties similar to the $\gamma$-casein isolated by previous workers (8, 14, 15). It contained very little impurity, based on starch gel electrophoresis in 7 M urea. The TS fraction showed, by zone electrophoresis at pH 8.6, a major band of mobility slower than that of $\gamma$-casein.

Three genetic types of $\beta$-casein, namely A, B, and C, have been found in milk by gel electrophoresis at alkaline pH in the presence of urea (1, 2, 30, 36). Type A of $\beta$-casein shows further complexity by electrophoresis at an acid pH, and it is subdivided into $A^1$, $A^2$, and $A^3$ variants (19, 31, 32).

Aschaffenburg (1) in 1961 found some evidence for genetic polymorphism in $\gamma$-casein, using paper electrophoresis. Recently, El-Negoumy (6) prepared $\gamma$-casein fractions from milk of individual cows by a modification of Hipp's method (14, 15). These fractions showed polymorphism by starch gel electrophoresis and the various bands were identified as $\gamma_1$ to $\gamma_5$.

Polymorphism in $\gamma$-casein has also been demonstrated, by using the high resolving power of disc gel electrophoresis, 4 M urea at pH 9.6, to examine caseins isolated from individual cows (12). To establish which bands would correspond to $\gamma$-casein, as defined by chromatography and described earlier (13), unfractionated casein was chromatographed on a DEAE-cellulose column, and a TS fraction was eluted at 0.005 M phosphate while the $\gamma$-casein was eluted at 0.02 M phosphate. Figure 1 shows the disc gel electrophoretic patterns of caseins typed with respect to $\beta$-casein, AC (Gel a), A (Gel b), AB (Gel c), and B (Gel d). The zones somewhat slower than the $\beta$-casein bands are designated $\gamma$-caseins. Their relative mobilities correspond in types to the $\beta$-caseins, with the exception of the AC type. With this sample, only $\gamma$-casein A is observed. The protein in the band designated R, with a mobility between that of $\gamma$-casein A and B, is not a $\gamma$-casein by the stated definition, since it is eluted from DEAE-cellulose in the TS fraction, with the TS- and S-caseins. These proteins will be discussed. They are called caseins because they occur in acid-precipitated casein, can be

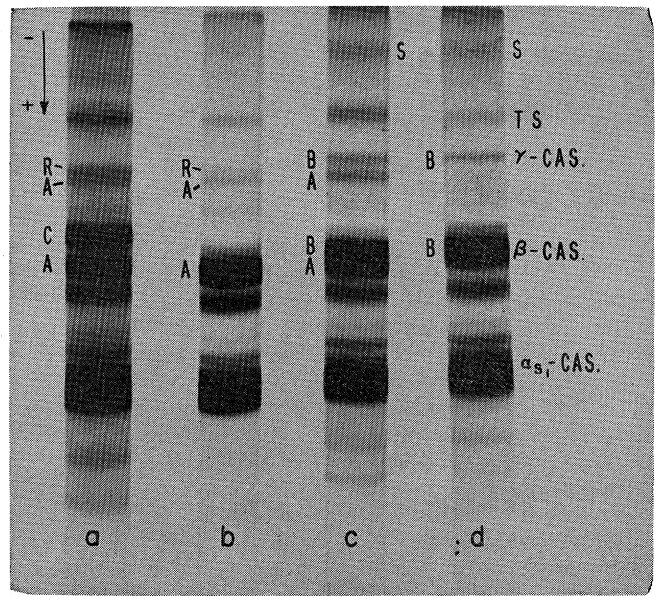


FIG. 1. Disc gel electrophoretic patterns, at pH 9.6 and 4 M urea, of caseins typed: a, $\beta$-casein AC, $\gamma$-casein A; b, $\beta$-casein A, $\gamma$-casein A; c, $\beta$-casein AB, $\gamma$-casein AB; d, $\beta$-casein B, $\gamma$-casein B.

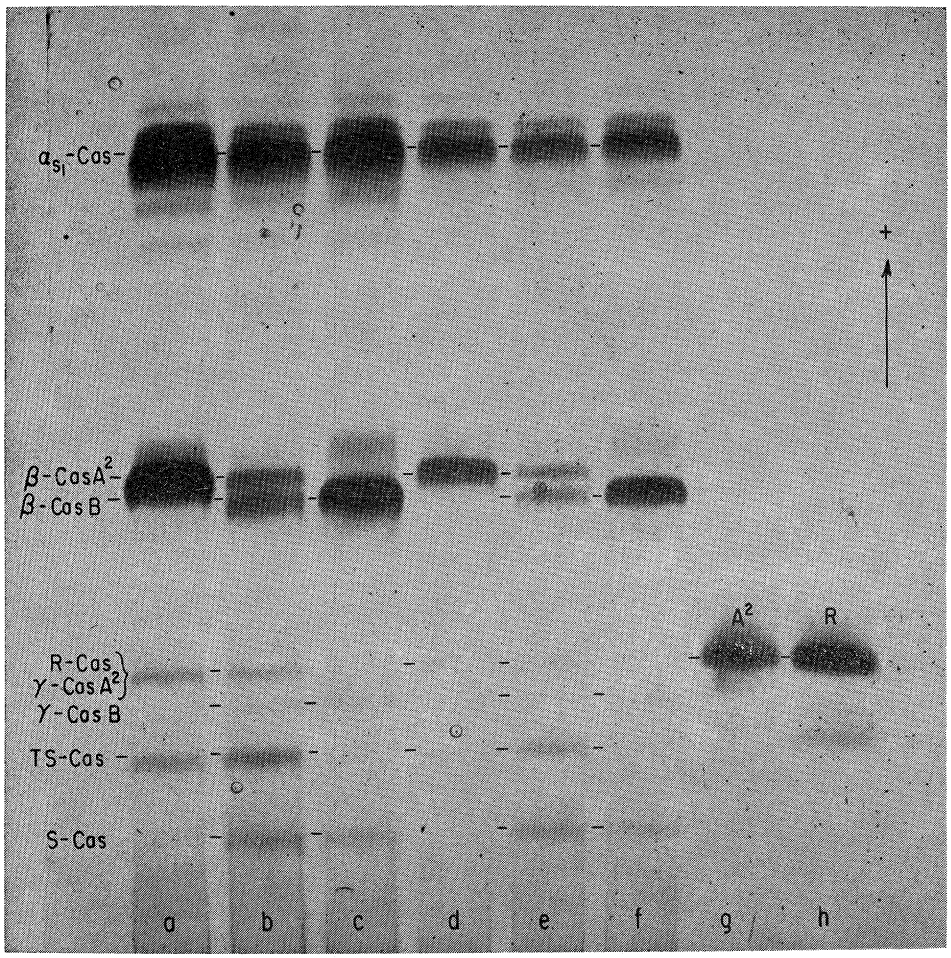


FIG. 2. Vertical gel electrophoretic patterns, at pH 8.7 and 4.5 M urea, of unfractionated caseins, together with $\gamma$-casein $A^2$ and R-casein. a, d, casein typed $\beta$-, $\gamma$-casein $A^2$; b, e, casein typed $\beta$-, $\gamma$-casein $A^2B$; c, f, casein typed $\beta$-, $\gamma$-casein B; g, $\gamma$-casein $A^2$; h, R-casein. The protein in d, e, f is half as concentrated as that in a, b, c.

identified in micellar casein by gel electrophoresis, and are similar to other caseins in content of high percentages of proline, glutamic acid, valine, and leucine, and in lack of cystine. It is not known, however, that they are phosphoproteins. Figure 2 shows vertical acrylamide gel electrophoretic patterns, pH 8.7 and 4.5 M urea, of unfractionated caseins and of the isolated $\gamma$-$A^2$ and R-caseins. Under these conditions, the S-, TS-, and $\gamma$-B caseins are clearly resolved, whereas the R- and $\gamma$-casein $A^2$ samples show the same mobilities. Gels a, b, and c represent samples of caseins typed $\beta$-, $\gamma$-casein $A^2$, $A^2B$, and B, respectively, while d, e, and f are of the same samples, but at one-half the concentration of protein. Zone g shows the band for $\gamma$-casein $A^2$, while h is that of the R-casein. Vertical gel electrophoretic patterns of caseins reduced with mercaptoethanol show that $\kappa$-casein and $\gamma$-casein bands are not clearly resolved. The $\kappa$-casein A band has a mobility slightly faster than that of $\gamma$-casein $A^2$, whereas the $\kappa$-casein B shows the same mobility as that of $\gamma$-casein B.

Two types of $\gamma$-casein, as determined by disc gels at pH 9.6, are designated A and B, and in all samples examined the $\gamma$- and $\beta$-caseins show the same types, with the exception of $\beta$-casein C. No $\gamma$-casein was found for the only two caseins available containing the homozygous $\beta$-casein C. The $\gamma$-caseins in unfractionated casein cannot be typed by disc gel electrophoresis at pH 4.3 and 8 M urea due to interference from other caseins. The $\gamma$-caseins isolated from casein typed $\beta$-casein $A^1$,

$A^2$, and $A^3$ show slightly faster mobilities than those of the corresponding $\beta$-caseins and the differences in relative mobilities are consistent with those found for the $\beta$-caseins (10). Figure 3 shows the disc gel electrophoretic patterns, pH 4.3, 8 M urea, of $\gamma$-caseins typed B, $A^1$, and $A^2$. The $\gamma$-casein $A^3$ sample, with a mobility less than that of $\gamma$-casein $A^2$, is not shown. A similar pattern is obtained (Fig. 4) when the $\gamma$-casein polymorphs are compared, using the vertical acrylamide gel electrophoretic method of Peterson and Kopfler (31) at pH 3.0 and 4.5 M urea. The $\gamma$-casein bands, under these conditions, show mobilities near that of $\alpha_{s1}$-casein. An unfractionated casein sample, together with a mixture of the isolated S-, TS-A, TS-B, and R-caseins, which will be discussed, are also included for comparison.

Purified samples of $\gamma$- and $\beta$-caseins were prepared from caseins typed either $A^2$ or B using chromatography on DEAE-cellulose columns (9, 39). Figure 5 shows disc gel electrophoresis, pH 9.6, 4 M urea, of the purified $\gamma$- and $\beta$-caseins isolated from the two casein types. Table 1 compares the number of amino acid residues found for the $A^2$ and B types of $\gamma$- and $\beta$-caseins. Values are based on molar ratios, taking glycine and alanine as 5 and 6, respectively, for the $\gamma$-caseins and 5 for the $\beta$-caseins. The amino acid substitutions for the $A^2$ and B types of both $\gamma$- and $\beta$-caseins (underlined in Table 1) are presumed to be arginine/serine and histidine/proline. Based on the genetic code, each substitution is consistent with one base substitution in the DNA molecule. The $\gamma$-casein molecule is larger than $\beta$-casein, having seven more proline residues and a net increase of seven other amino acids. Aside from proline, the other amino acids of the $\gamma$- and $\beta$-caseins differ by no more than two residues. The similarities in relative mobilities of the $\gamma$-casein and $\beta$-casein polymorphs, together with the common substitutions exhibited by the $A^2$ and B types, suggest a close relationship in the synthesis of $\gamma$- and $\beta$-casein.

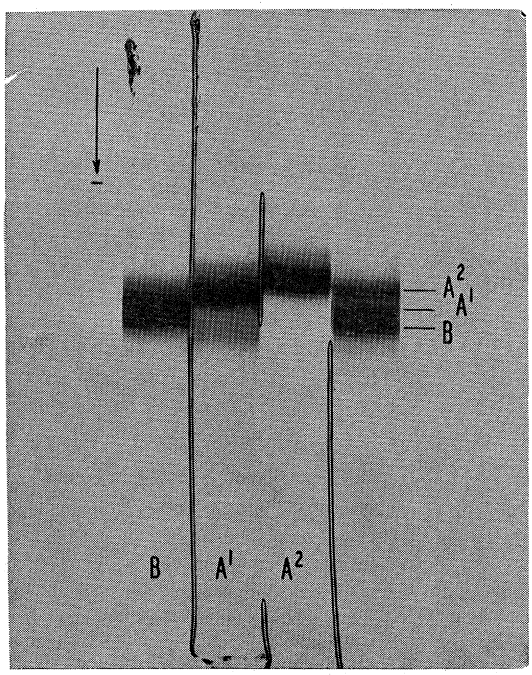


FIG. 3. Disc gel electrophoretic patterns, at pH 4.3 and 8 M urea, of the isolated $\gamma$-caseins typed B, $A^1$, and $A^2$. A mixture of all three proteins is shown in one gel.

*R-, S-, and TS-caseins.* The TS fraction, as mentioned earlier, is that fraction of casein eluted from a DEAE-cellulose column at pH 8.3 with the starting buffer of 0.005 M phosphate. It contains the bands referred to in Figure 1 as S-, TS-, and R-casein, together with other caseins, including some $\kappa$-casein. Reasonably pure preparations of these proteins can be obtained by rechromatography of the TS fraction on DEAE-cellulose, either with or without urea. The $A^2$ type yields R-casein and TS-casein A; the B type gives S-casein and TS-casein B. Both TS-A and TS-B samples show the same mobility by disc gel electrophoresis at pH 9.6, 4 M urea (10, 11). However, at pH 4.3 and 8 M urea, the TS-A has a faster mobility than TS-B, Figure 6. The Gels a and c show S- and TS-casein B obtained from casein of the B type shown in Gel f. The Gels b and d show TS-casein A and R-casein obtained from the $A^2$ type casein shown in Gel g. The band in Gel f, corresponding in mobility to R-casein, is that of $\gamma$-casein B. Casein samples of the AB type show by disc gel electrophoresis both the TS-A and TS-B caseins. Vertical gel electrophoresis at pH 3.0 and 4.5 M urea of the partially purified minor caseins and an unfractionated casein sample typed $\beta$-, $\gamma$-casein $A^2$ are shown in Figure 7. Here the S-, TS-A, TS-B, and R-caseins are better separated from the $\gamma$- and other caseins than in the disc gel runs (Figure 6). This was also shown in Figure 4, b. The casein sample (Slot a) in both Figures 4 and 7 shows a faster band corresponding to TS-A and a faint band of slower mobility that corresponds to that of the R-casein.

Table 2 compares the various types of minor proteins with the $\beta$-casein types as found in casein of individual cows. For the caseins

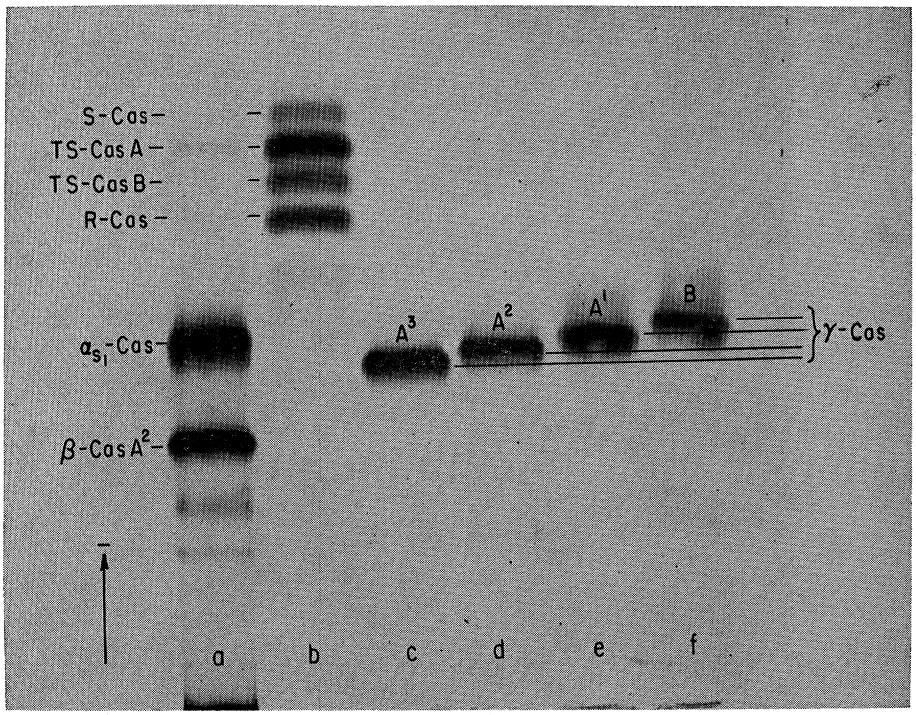


FIG. 4. Vertical gel electrophoretic patterns, at pH 3.0 and 4.5 M urea, of the γ-casein polymorphs, together with unfractionated casein and a mixture of the isolated S-, TS-A, TS-B, and R-caseins. a, casein typed β-, γ-casein $A^2$; b, S-, TS-A, TS-B, and R-casein: c, γ-casein $A^3$; d, γ-casein $A^2$; e, γ-casein $A^1$; f, γ-casein B.

examined by alkaline typing, those containing β-casein A, B, and C also have the corresponding γ-casein A and B but no C. As mentioned earlier, the TS-caseins give a single band of the same mobility at alkaline pH values. Samples typed β-casein B contain S-casein but no R-casein, whereas all other β-casein samples typed $A^1$, $A^2$, $A^3$, and C contain the R-casein but no S-casein. The γ-caseins in unfractionated casein, as previously stated, cannot be typed at acid pH, due to interference from other proteins. The limited number of γ-caseins isolated do show relative mobilities similar to those found in the corresponding β-caseins $A^1$, $A^2$, and $A^3$. The TS-caseins A and B in unfractionated caseins can be identified by electrophoresis at acid pH. The TS-A in samples typed β-casein $A^3$ appears to differ from the other A types, since it shows two bands of different mobility from that of TS-A and TS-B. Preliminary amino acid analysis and the disc gel electrophoretic data indicate that the TS-caseins from samples typed A and B, like the γ-caseins, are polymorphic (10) and, furthermore, that they contain more proline than any other caseins studied. Based on disc gel electrophoresis, the R- and S-caseins could also be polymorphs of the same protein. Further analyses are in progress to determine if this is so.

No attempt has been made to identify the minor slow-moving casein components by num-

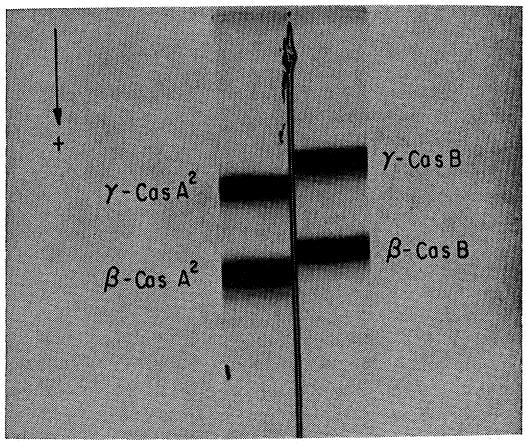


FIG. 5. Disc gel electrophoretic patterns, at pH 9.6 and 4 M urea of purified γ- and β-caseins isolated from caseins typed β-, γ-casein $A^2$, and β-, γ-casein B.

TABLE 1. Comparison of amino acid residues of $\gamma$- and $\beta$-caseins typed $A^2$ and B (9).

| | $\gamma$-$A^2$ | $\gamma$-B | $\beta$-$A^2$ | $\beta$-B |
|---|---|---|---|---|
| | Residues per molecule | | | |
| Lysine | 12 | 12 | 11 | 11 |
| Histidine | 6 | 7 | 5 | 6 |
| Arginine | 3 | 4 | 4 | 5 |
| Aspartic Acid | 9 | 9 | 9 | 9 |
| Threonine | 10 | 10 | 9 | 9 |
| Serine | 13 | 12 | 15 | 14 |
| Glutamic Acid | 39 | 39 | 39 | 39 |
| Proline | 41 | 40 | 34 | 33 |
| Glycine | 5 | 5 | 5 | 5 |
| Alanine | 6 | 6 | 5 | 5 |
| Valine | 20 | 20 | 18 | 18 |
| Methionine | 7 | 7 | 6 | 6 |
| Isoleucine | 8 | 8 | 10 | 10 |
| Leucine | 23 | 23 | 21 | 21 |
| Tyrosine | 5 | 5 | 4 | 4 |
| Phenylalanine | 11 | 11 | 9 | 9 |
| Tryptophan | 1 | 1 | 1 | 1 |
| Mol wt [a] | 25,020 | 25,130 | 23,590 | 23,700 |

[a] Calculated from composition shown, including phosphorus and, in the case of $\gamma$-casein, one residue of hexose.

bering the bands obtained by starch gel electrophoresis according to the system developed by Wake and Baldwin (40), since the minor caseins described here were identified using the disc gel electrophoretic technique. It is probable that some of these caseins correspond to Bands 0.18, 0.34, and 0.41 found, as mentioned earlier, in the $\gamma$-fraction of Mackinlay and Wake (26), since this fraction was eluted first from a DEAE-cellulose column. It appears that most of the recent studies on casein have made use of vertical acrylamide gel electrophoresis. For this reason, and for comparison with the disc gel technique, the patterns obtained by both methods have been shown where possible.

$\beta$-Casein contains four or five moles of phosphorus per mole of casein, depending on the type (37), while the $\gamma$-caseins $A^2$ and B contain only one mole of phosphorus (9). This relatively small amount of phosphorus in $\gamma$-casein is probably reflected in its slower electrophoretic mobility at an alkaline pH, as compared with that of $\beta$-casein. It is not inconceivable, then, that other minor proteins such as the S- and TS-caseins, with mobilities less than that of $\gamma$-casein, might contain no phosphorus. This would be inconsistent with the definition of casein as a phosphoprotein, even though preliminary amino acid analyses indicate that these minor proteins belong to the $\beta$- and $\gamma$-casein family, since they contain, like $\beta$- and $\gamma$-casein, no cystine but relatively large amounts of glutamic acid, proline, valine, and leucine.

*$\alpha_{s2}$-, $\alpha_{s3}$-Caseins and 0.86 casein.* A casein corresponding to Band 0.86, as already mentioned, was found by Mackinlay and Wake (26) to disappear when casein was reduced and alkylated prior to zone electrophoresis. This protein has now been isolated by Hoagland and Kalan (16). It was partially purified by chromatography on DEAE-cellulose. The fraction enriched in protein corresponding to Band 0.86 was then fractionated by preparative polyacrylamide electrophoresis. The purified protein on reduction with mercaptoethanol has a greater mobility than the unreduced protein by zone electrophoresis at pH 9.2 and, as might be expected, contains cystine. This protein differs from the $\alpha_{s1}$-, $\beta$-, and $\gamma$-caseins, which contain no cystine. Also, it is relatively low in proline content but like other caseins high in glutamic acid. The casein corresponding to Band 0.86 is a phosphorus-containing protein.

Casein contains two minor components which show mobilities slightly slower than that of $\alpha_{s1}$-casein by gel electrophoresis at alkaline pH. Thompson (34) isolated these proteins by urea fractionation of casein, then by alcohol-

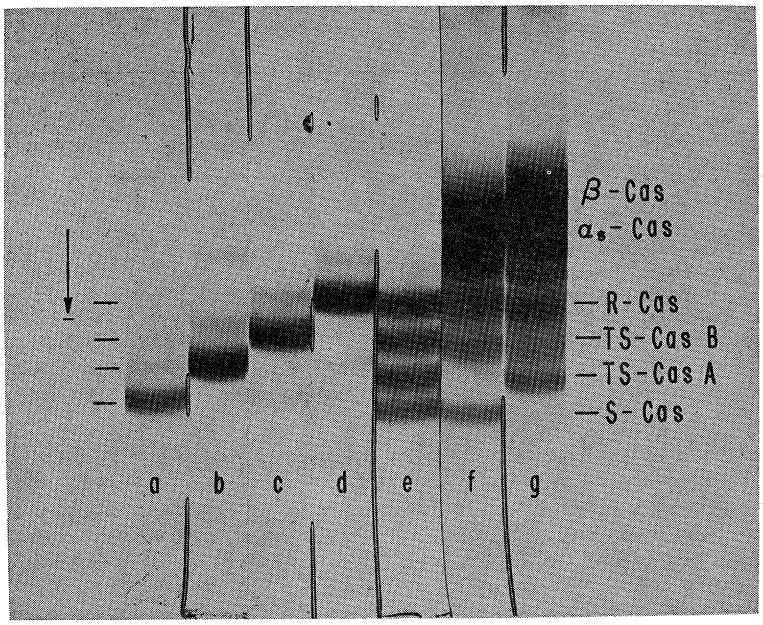


FIG. 6. Disc gel electrophoretic patterns, at pH 4.3 and 8 M urea, of partially purified S-, TS-A, TS-B, and R-caseins, together with the unfractionated caseins. a, S-casein; b, TS-casein A; c, TS-casein B; d, R-casein; e, a mixture of S-, TS-A, TS-B, and R-casein; f, casein typed $\beta$-, $\gamma$-casein B; g, casein typed $\beta$-, $\gamma$-casein $A^2$.

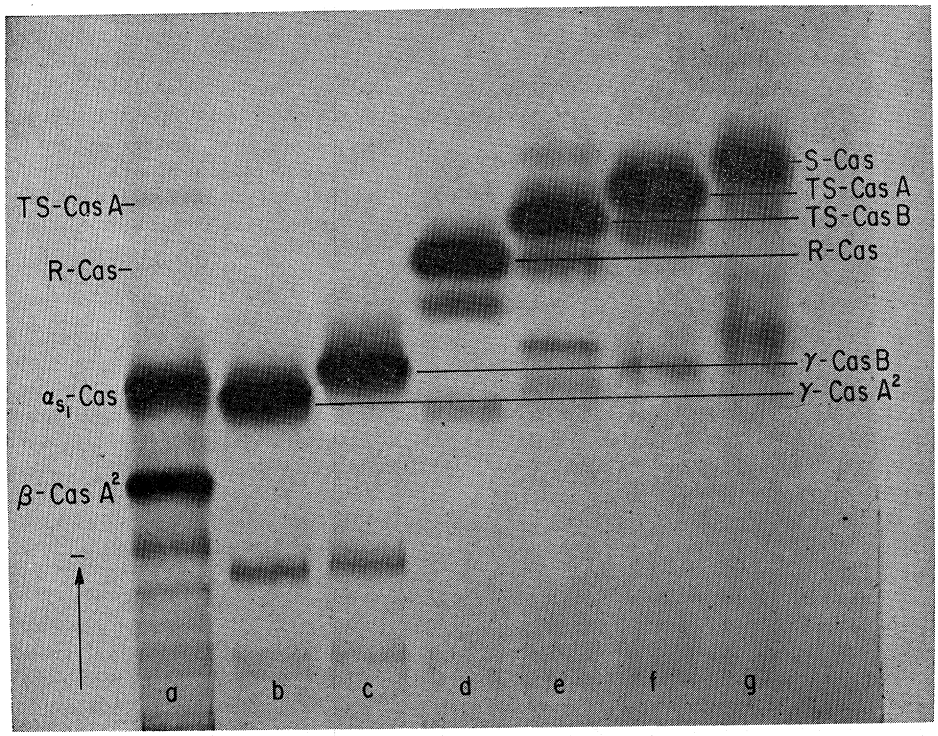


FIG. 7. Vertical gel electrophoretic patterns at pH 3.0 and 4.5 M urea, of partially purified minor caseins, together with an unfractionated casein sample. a, Unfractionated casein typed $\beta$-, $\gamma$-casein $A^2$; b, $\gamma$-casein $A^2$; c, $\gamma$-casein B; d, R-casein; e, TS-casein B; f, TS-casein A; g, S-casein.

TABLE 2. Types of $\beta$-, $\gamma$-, TS-, R-, and S-caseins found in casein samples obtained from individual milks.

| $\beta$-Casein | $\gamma$-Casein | TS-Casein | R- or S-Casein |
|---|---|---|---|
| $A^1$ | $A^1$ | A | R |
| $A^2$ | $A^2$ | A | R |
| $A^3$ | $A^3$ | $A^{3a}$ | R |
| B | B | B | S |
| C | — | A | R |

[a] Preliminary evidence suggests that this sample shows a mobility slightly different from other types designated A, and that it contains another polymorph of mobility slower than that of TS-B. The TS-$A^3$ designation is temporary, until further studies can be made on this protein.

ammonium acetate fractionation and finally by DEAE-cellulose column chromatography. Like $\alpha_{s1}$-casein, they are calcium-sensitive and are stabilized against calcium precipitation by $\kappa$-casein. They were named $\alpha_{s2}$- and $\alpha_{s3}$-casein, in order of their decreasing mobility. They correspond in mobility to the zone numbers 1.04 and 1.00 of Wake and Baldwin's patterns (40). A comparison of the amino acid composition of the $\alpha_{s2}$- and $\alpha_{s3}$-caseins indicates that they both contain cysteine or cystine, but show small differences in lysine, aspartic acid, glutamic acid, proline, and isoleucine. They differ significantly from $\alpha_{s1}$-casein in composition, and more closely resemble the casein of Zone 0.86 with respect to the presence of cystine and a relatively low amount of proline. There is some evidence that the protein of Zone 0.86 represents the oxidized form of $\alpha_{s2}$- (Zone 1.04) and $\alpha_{s3}$ (Zone 1.00) caseins.[1] If this is true, it would be of interest to know whether these proteins could be substrates for the enzyme, sulfhydryl oxidase, recently found in milk by Kiermeier et al. (20, 21).

*Proteose-peptone fraction.* When skimmilk is heated at 95 to 100 C for 30 min, then adjusted to pH 4.6 by addition of acid, most of the whey proteins are denatured by the heat treatment and precipitate with the casein, leaving a proteose-peptone fraction in solution (38). A recent review of this subject has been made by McKenzie (27). Larson and Rolleri (24) determined by moving-boundary electrophoresis that the proteose-peptone fraction consists of three components designated 3, 5, and 8, of increasing mobility. Component 8 showed a mobility at pH 8.6 faster than that of serum albumin; Component 5 was between $\alpha$-lactalbumin and $\beta$-lactoglobulin; and Component 3 had a mobility slightly slower than that of $\alpha$-lactalbumin. It was not known whether these proteins were present in native milk or whether they were artifacts resulting from the heat treatment, as thought by early workers in the field.

Brunner and Thompson (5) compared several methods of isolating the proteose-peptone-like fractions from the same milk. All preparations were heterogeneous by moving-boundary electrophoresis, although they appeared to contain a common major component, and they all contained carbohydrate and a relatively high amount of phosphorus. On ultracentrifugation, two boundaries were observed, with sedimentation coefficients of about 0.8 S and 2.8 S.

Indications are that electrophoretic Component 5 is a native protein in milk, since Jenness (17) has isolated a protein resembling this component using mild fractionation procedures. Fractionation was accomplished by salting out the casein, lactoperoxidase, and other proteins by saturation of raw skimmilk with sodium chloride. The precipitate was redispersed and the casein precipitated by addition of acid to pH 4.6. An ion exchange resin was then used to adsorb lactoperoxidase from the protein that remained in solution, then Component 5 was selectively precipitated by careful adjustment of the pH to about 4.5. Component 5 was 90% pure, based on area measurements of its electrophoretic pattern obtained at pH 8.6, but somewhat more heterogeneous at pH 2.3. The electrophoretic mobility of Component 5 was $-4.5 \times 10^{-5}/cm^2/V^{-1}/sec^{-1}$ at 0.1 ionic strength and pH 8.6 in veronal buffer. It contains about 1.2% phosphorus, is soluble in calcium chloride at 0.02 and 0.2 M concentrations, and is not clotted by rennin, either in the presence or in the absence of calcium.

Brunner and co-workers (22, 23, 29) more recently have isolated and further characterized

[1] M. P. Thompson and P. D. Hoagland, personal communication.

Components 3, 5, and 8. Component 3, a whey protein by their designation, was isolated from both raw and heated skimmilk by a combination of salting-out with ammonium sulfate and preparative gel electrophoresis (29). The protein gave a single zone by polyacrylamide gel electrophoresis and showed, by moving out boundary electrophoretic data, an isoelectric point of pH 3.7. It had a sedimentation coefficient of 3.4 S and a molecular weight of 125,000 as determined by sedimentation-equilibrium. It contained 17.2% carbohydrate, consisting of 7.2% hexose (galactose and mannose), 6.0% hexosamine (glucosamine and galactosamine), 1.0% fucose, 3.0% sialic acid; it also contained 0.5% phosphorus. Component 3 contained small amounts of aromatic amino acids and methionine, but large amounts of aspartic and glutamic acids and no cystine or cysteine.

Component 8 was isolated from the proteose-peptone fraction of acid whey and from both isoelectric and micellar casein complexes (22). This component is found associated with κ-casein and may be identical to λ-casein (25). It contains carbohydrate and 1.2% phosphorus. In subsequent work Component 8 was resolved by gel filtration into two heterogeneous zones, designated 8-fast and 8-slow (23). Both are phosphoglycoproteins, since they contain 0.97 and 3.29% phosphorus, respectively, and varying amounts of hexose, hexosamine, and sialic acid. Component 5 was also isolated, although the fractionation procedure was not given. Component 5 showed a doublet on polyacrylamide gel at pH 8.6. The Component 8 fractions contain high concentrations of glutamic acid, aspartic acid, serine, and threonine, but no cysteine or cystine. Sedimentation coefficients $S_{20,w}$, for Components 5, 8-fast, and 8-slow are 1.22 S, 0.78 S, and 1.35 S. The corresponding molecular weights as determined by sedimentation-equilibrium are 14,300, 4,100, and 9,900. Since Components 5, 8-fast, and 8-slow are found in micellar casein as well as the serum, Brunner and co-workers (5) suggest that these proteins are caseins.

Ganguli et al. (7) found that the proteose-peptone fraction amounts to about 173 mg per 100 ml of milk and that this value is greater in colostrum. They determined the amino acid composition of the proteose-peptone fraction and also showed that cystine and serine were the only N-terminal amino acids present.

*Acid glycoproteins.* Bezkorovainy (3, 4) found that the acid glycoproteins from bovine blood serum, colostrum acid whey, and milk acid whey were adsorbed by DEAE-cellulose at pH 4.3 and low ionic strength. The acid glycoproteins could then be eluted at pH 5.0 with buffers of increasing ionic strength. CM-cellulose was also used for further fractionation. Two major fractions, designated M-1 and M-2, were isolated and studied. The blood serum was shown to contain mostly orosomucoid in the M-1 fraction and an M-2 glycoprotein. A fraction containing orosomucoid and M-2 glycoprotein was also isolated from bovine colostrum, while milk contained no orosomucoid and only trace amounts of the M-2 glycoprotein. However, both milk and colostrum showed in the M-1 fraction a phosphoglycoprotein of low absorption at 280 mμ. Its carbohydrate and phosphorus content and its electrophoretic mobility at pH 4.5 and 8.6 was similar to that of the proteose-peptone fraction and κ-casein. The phosphoglycoprotein differed from κ-casein, as indicated by differences in its sedimentation behavior and amino acid composition. The phosphoglycoprotein contained 3.1% hexose, 2.4% N-acetyl hexosamine, and 4.0% sialic acid. Its phosphorus content was 0.44%.

The M-1 acid glycoprotein fractions obtained from the milk and colostrum of individual cows were further fractionated by gel filtration and chromatography on DEAE-Sephadex, to give samples that were homogeneous by ultracentrifugation and moving-boundary electrophoresis. These samples showed electrophoretic mobilities at pH 8.6 of $-5.7$ to $-5.85 \times 10^{-5}/cm^2/V^{-1}/sec^{-1}$ and sedimentation coefficients of 1.03-1.30 S, with molecular weights of from 9,500 to 12,800. Since they contained several N-terminal amino acids and in some samples showed several bands on gel electrophoresis, they may consist of a series of related molecular species. The amino acid and carbohydrate composition of M-1 glycoproteins from individual milks was also variable. The acid glycoproteins contained relatively large amounts of glutamic acid, proline, threonine, and isoleucine, while the basic amino acids were low. Values for cystine were not reported. Like the caseins, they showed high negative specific rotations. The colostrum M-1 glycoprotein fraction contained galactose, glucosamine, galactosamine, and sialic acid. Similar carbohydrates were found in the milk glycoprotein fraction, with the exception that the type of hexosamines was not determined. The sialic acid was easily removed by viral neuraminidase. Phosphorus values were not reported for these preparations.

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